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THE EFFECTS OF ACUTE GAMMA IRRADIATION ON THE BRINE SHRIMP, *ARTEMIA*. III. MALE F_1 REPRODUCTIVE PERFORMANCE FOLLOWING PATERNAL IRRADIATION OF MATURE SPERM [1]

RICHARD D. SQUIRE

Biology Department, Long Island University, Brooklyn, New York 11201

It was reported in the first paper of this series (Squire and Grosch, 1970) that the F_1 sons of irradiated males are much more sterile than are the irradiated males themselves. However, data were not available for quantitative analysis at that time.

Population studies have indicated two major patterns of response following acute irradiation of mature gametes. The majority of the species studied to date demonstrate a greater degree of sterility in the irradiated generation than in subsequent descendants. The irradiated generation may thus serve as an immediate assay for reproductive damage. In the second pattern of radiation response, the descendants of irradiated animals are much more sterile than is the irradiated generation itself. This second pattern of response has been only recently explored, and is correlated with chromosome structure (see La Chance *et al.*, 1970). Instead of having a single region of centromeric activity per chromosome (monokinetic chromosome structure), these latter organisms have many regions of centromeric activity per chromosome (diffuse centromeres, holokinetic or polycentric chromosomes). The holokinetic nature of the chromosome is expected to reduce acentric fragment loss following irradiation, and hence reduce the degree of induced P_1 sterility associated with such induced genetic dominant lethal events. The surviving offspring, however, may contain many simple and complex chromosomal rearrangements. After meiosis, this is expected to result in significantly increased F_1 sterility due to the production of genetically unbalanced gametes which cause lethality in the F_2. The excellent paper by LaChance, Degrugillier and Leverich (1970) discusses the cytogenetic aspects in much greater detail and provides a review of the earlier papers dealing with holokinetic chromosome structure. The vast majority of these radiation studies in which increased F_1 sterility was found utilized lepidopteran, homopteran or hemipteran insects.

Stefani (1963c) reported that *Artemia* has polycentric chromosomes and indicated their possible interest to radiation biologists. Stefani's studies were often based upon early blastomere divisions, but he has examined meiotic stages as well (Stefani, 1961, 1963a, 1963b, 1964, 1967). In their extensive review, Metalli and Ballardin (1970–72) question Stefani's interpretation, stating that they have found clear primary constrictions in a metacentric position when studying larval stages, oocytes and male meiosis. Based on electron microscope examinations,

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Fautrez-Firlefyn and Roels (1968) consider *Artemia* chromosomes to have a diffuse kinetochore.

The present study therefore provides indirect evidence supporting the holokinetic nature of the *Artemia* chromosome. The small numerous chromosomes ($2n = 42$) of the of the *Artemia* complement make cytogenetic analysis of induced aberrations rather difficult, but such a study has begun.

Materials and Methods

Culture techniques were essentially the same as reported earlier (Squire and Grosch, 1970). Male brine shrimp were hatched from commercially obtained California cysts, and were approximately 5–6 weeks old at the time of exposure. Doses were 0, 1, 2, 3.5, 5, or 10 kR acute gamma radiation using a Gammator-50 (507 R/min, 137-Cs).

Irradiated and control males were individually pair mated to homozygous white eyed females (Xw/Yw). This use of a recessive genetic marker from the female, and a dominant allele from the male proves the paternity of the offspring and removes the necessity of using unmated females. (There is no sperm storage in this species and each brood is produced by a separate mating.) In general, the F_1 were raised to maturity and scored for survival to adulthood and sex ratio. Several F_1 sons (3 if available) were selected from each P_1 male and individually pair mated to comparable females which lacked any radiation in their ancestry. (Thus the control "F_1" females became the mates for the F_1 males of control and of irradiated ancestry.) Each F_1 female was either unmated or virgin when used. Pedigree records of each mating were maintained. Care was exercised to avoid any brother-sister matings, since inbreeding depression is extremely pronounced in this species (Squire, 1969), and would be confounded with induced sterility. It is important to note that the terms F_1 and F_2 as used in this paper refer to outcrossed rather than inbred generations.

These experiments were conducted over a period of $2\frac{1}{2}$ years and hence encompassed some variation in environmental conditions. Temperatures ranged from 70° to 85° F, but were usually maintained at 78° to 82° F by use of a room airconditioner. These variations are of little importance since treated animals were always concurrent with their controls. Repeated tests at 0, 5 and 10 kR gave essentially identical results. Although full P_1 and F_1 reproductive data were obtained at some selected dose levels, the magnitude of data precluded such an analysis at others. The hatchability of cysts obtained from P_1 and F_1 animals was usually low, whether or not there was a history of irradiation. This low hatchability limited the kinds of comparisons which could be made. Survival to adulthood and sex ratio data were obtained by placing 50 (if available) freshly produced nauplii from a single viviparous brood in a quart jar of brine. The sample was never smaller than 25 nauplii, and was restricted to a maximum of 50 nauplii in order to minimize the selective effects of crowding. Different samples represented the products of different pairs.

Data were restricted to young which were produced during the first 11 days (8 days for 1 kR) after paternal irradiation in order to further ensure the probability that they were derived from cells which were spermatozoa at the time of irradiation (Squire and Grosch, 1970).

TABLE I

Survival to adulthood and sex ratios of F_1 Artemia after a paternal dose of 5 or 10 kR

Dose (kR)	No. of young produced	Survival to adulthood				Sex ratio % males
		Males	Females	Total	%	
0	754	292	288	580	76.9	50.3
5	593	196	140	336	56.7	58.3
10	539	176	121	297	55.1	59.3
X^2 comparison				88.1 $P < 0.005$		9.24 $P < 0.01$

It should be noted that the *Artemia* utilized in this study had different genetic backgrounds than did the animals used earlier (Squire and Grosch, 1970; Squire, 1970). Some of these genetic differences were probably reflected in differences in life span and fertility data which become apparent when comparing experiments. However, the similarities in response are much more pronounced than are the differences.

OBSERVATIONS

5 and 10 kR

On the basis of 1886 F_1 shrimp, there is a significant 27 per cent reduction in F_1 survival to adulthood after a paternal dose of either 5 or 10 kR. A significant increase in male sex ratio was also observed (Table I). Two replicates were run and the data were pooled since no differences were found between replicates. All Chi-square contingency tests were conducted according to Steel and Torrie (1960, pages 370–372).

Three F_1 males were selected from each brood when available, and individually pair mated to untreated females. Each pair mating was scored throughout the entire life span of the F_1 male. F_1 males were almost completely sterile after a paternal dose of either 5 or 10 kR. The paternal dose, number of F_1 pairs, number of fertile F_1 males, total number of viviparously produced F_2 progeny, and average F_1 male adult life span are indicated in Table II. No F_2 progeny were produced ovoviviparously after paternal doses of 5 or 10 kR.

TABLE II

F_1 male sterility and average adult life span after a paternal dose of 5 or 10 kR

Dose (kR)	No. of F_1 pairs	No. of fertile F_1 males	Total no. of F_2 progeny	F_1 male adult life span (days)	
				$\bar{X} \pm$ S.E.	t
0	12	12	15,006	88.4 ± 16.5	
5	12	4	51	40.4 ± 4.9	2.79*
10	14	2	9	66.6 ± 10.6	1.11NS

* Significant at 0.02.

TABLE III

F_1 male reproductive data (scored for 35 days) after a paternal dose of 1 kR

Criterion	0 kR	1 kR
Fraction of pairs surviving 35 days	20/30	19/27
Average number of viviparous broods per surviving pair	4.30	3.00
Average number of ovoviviparous broods per surviving pair	2.15	3.10
Number of sterile males in surviving pairs	0	1
Average number of young per viviparous brood from surviving pairs	96.20	80.23
Average per cent cyst hatchability on a per pair basis	11.7	15.9

1 kR

Since 5 and 10 kR were sufficient to induce almost complete F_1 male sterility, a lower dose of 1 kR was next investigated. Survival to adulthood was 93.8 per cent in each group and there was no effect on sex ratio. F_1 male life span and reproductive data were kept for 35 days and failed to detect any effect of P_1 irradiation (Table III).

2 kR

A new experiment was therefore initiated using a paternal dose of 2 kR. A preliminary analysis failed to detect any effect of paternal irradiation on F_1 male reproductive performance, and this experiment was abandoned.

3.5 kR

A final experiment was conducted using a paternal dose of 3.5 kR. This dose resulted in an 11 per cent reduction in survival to adulthood. The change in sex ratio was not significant with the sample size used (Table IV). This dose resulted in a 44 per cent increase in the number of sterile pairs (Table V). Several of the fetrile males were characterized by broods which repeatedly contained incompletely developed embryos in addition to normal-appearing nauplii. Pairs were scored until (a) they had produced several viviparous broods, (b) they had produced at least three ovoviviparous broods, or (c) the male died. It was initially

TABLE IV

Survival to adulthood and sex ratios of F_1 Artemia *after a paternal dose of 3.5 kR*

Dose (kR)	No. of young produced	Survival to adulthood				Sex ratio % males
		Males	Females	Total	%	
0	266	107	109	216	81.2	49.5
3.5	647	261	204	465	71.9	56.1
X^2 comparison				8.18 $P < 0.005$		2.32NS $P > 0.10$

TABLE V

Number of sterile pairs after a paternal dose of 3.5 kR

Dose (kR)	Fertile pairs		Sterile pairs		Total
	Number	%	Number	%	
0	16	69.6	7	30.4	23
3.5	14	30.4	32	69.6	46
Total	30	43.5	39	56.5	69

$X^2 = 8.03$. $P < 0.001$.

assumed that cyst analysis would give a reliable estimate of fertility in the absence of viviparous reproduction. However, cyst hatchability was too low in this experiment to use as a criterion of damage, and probably gave an inflated estimate of the actual number of sterile males in both the controls and the descendants of irradiated males. Nevertheless, the per cent reduction in the number of fertile males (treated *vs.* control) is probably a meaningful estimate. The low cyst hatchability observed here is believed due to a genetic deterioration of the white eye unirradiated stock which resulted in improper cyst formation. Many females consistently produced translucent cysts or unencysted embryos which subsequently failed to develop.

DISCUSSION

The current survival to adulthood and sex ratio data after a paternal dose of 5 or 10 kR are based on larger samples than those of Squire and Grosch (1970). In our earlier paper, we noted a non-significant trend toward increased male sex ratio. This trend reached statistical significance in the present study. The shift in sex ratio could be due to reduced female viability. The female is the heterogametic sex in *Artemia,* and perhaps the X chromosome is partially hemizygous for viability genes. Sufficient genetic data is lacking for evaluating this hypothesis at the present time and alternative hypotheses could be evoked.

A paternal dose of 5 or 10 kR resulted in a 27 per cent reduction in F_1 survival to adulthood. No difference was observed between the 5 and 10 kR treatments, and this failure cannot be explained at the present time. Survival to adulthood was reduced by 11 per cent after 3.5 kR, but was not affected at lower doses.

The F_1 male sterility and average adult life span data confirm and extend the qualitative observations of Squire and Grosch (1970). A paternal dose of 5 or 10 kR results in virtually complete sterility of surviving F_1 males. A paternal dose of 1 kR had no effect on F_1 survival to adulthood, sex ratio or male reproductive performance. Incomplete data indicated that the same holds true for a paternal dose of 2 kR. A paternal dose of 3.5 kR resulted in a 44 per cent reduction in the number of fertile males, and also reduced the degree of fertility of at least some of the reproducing males (*i.e.,* partial sterility).

An unexpected finding in these experiments is the relatively narrow dose range sufficient to span the gap from no demonstrable F_1 male sterility (2 kR or more?)

on the one hand, to virtually complete F_1 male sterility (5 kR or less) on the other. It should be emphasized that even this higher dose of 5 kR was without obvious effect on the initial fertility of the irradiated males themselves, although it did reduce F_1 post natal viability. F_1 survival to adulthood measures viability during development of the free-swimming nauplius into an adult, and is thus a measure of lethal events which occur relatively late in development. Early embryonic deaths would be manifest as aborted embryos which failed to develop into free-swimming nauplii. Such embryos were found in the F_2 after 3.5 kR, but not in the F_1. Delayed F_1 mortality and the greater degree of F_1 male sterility (as compared to their fathers) are characteristic of organisms with holokinetic chromosomes, but diametrically opposed to results obtained with organisms which have monokinetic chromosomes.

In organisms which contain holokinetic chromosomes it has been repeatedly demonstrated that the F_1 sterility is associated with genetically unbalanced gametes which result from improper meiotic segregation of heterozygous translocation complexes. An additional factor may be secondary chromosome fragmentation and fragment loss (Brown and Wiegmann, 1969; LaChance *et al.*, 1970; Nordenskiold, 1963; North and Holt, 1968). Although such species are able to transmit small fragments through many mitotic divisions, there is increasing evidence supporting the view that many fragments which are retained in successive mitotic divisions are nevertheless eliminated during meiosis in the mealy bug, *Planococcus* (Brown and Wiegmann, 1969), and the silk worm, *Bombyx* (Inagaki and Nakao, 1970). However at least some such fragments are retained over successive generations in the large milkweed bug, *Oncopeltus* (LaChance *et al.*, 1970), and the wood rush, *Luzula* (Nordenskiold, 1963). It is not yet clear whether such a preferential elimination is in some way caused by a difference in the way in which the spindle fibers attach to the chromosomes during meiosis as compared to mitosis (Braselton, 1971; Buck, 1967; Comings and Okada, 1972).

In the current study, no attempt was made to distinguish between chromosomal sterility and possible abnormal histological, physiological or behavioral factors which might also be involved in F_1 sterility.

It is important to note that in organisms with holokinetic chromosomes, the effects of radiation exposure may be much more pronounced in the F_1 than in the irradiated generation itself. It is therefore quite possible for a natural population to receive a radiation dose which will result in population collapse of the subsequent generation. The usual methods of sampling field populations might fail to detect such a change before irreversible damage occured. This implication has become well known to insect-control researchers, but has remained largely unnoticed by many other investigators. Animals with holokinetic chromosomes include several groups of insects (Lepidoptera, Hemiptera, Homoptera, Odonata), brine shrimp (*Artemia*), and the Brazilian scorpion (*Tityus*). Many of these species are of considerable ecological and economic importance.

Summary

Male brine shrimp (*Artemia*) were hatched from commercially obtained Californian cysts and irradiated as adults with acute doses of 0, 1, 2, 3.5, 5 or 10 kR

gamma rays. Each male was individually pair mated to a white eye female. The F_1 were raised to maturity and scored for survival to adulthood, sex ratio, and F_1 male reproductive performance. There was a significant decrease in F_1 survival to adulthood after paternal doses of 3.5, 5 and 10 kR, but not at lower doses. A significant increase in male sex ratio was observed after 5 and 10 kR, and these males were almost completely sterile. A paternal dose of 3.5 kR resulted in a 44 per cent decrease in the number of fertile males and some fertile males were semi-sterile. No apparent effect was observed after 1 or 2 kR.

These data support the proposed holokinetic nature of the *Artemia* chromosome. They also demonstrate that the observable effects of radiation may be much more extreme in the F_1 animals than in the irradiated generation itself. This observation has important implications when assaying the effects of radiation on natural populations.

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